The effect of the level of dietary protein, carbohydrate and fat on urea kinetics in young children during rapid catch-up weight gain

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The kinetics of urea metabolism were measured in children recovering from severe malnutrition. For a period of up to 10 d they received one of four diets which provided 711 kJ (170 kcal)/kg per d. Two groups received a diet with a high protein:energy (P:E) ratio of 10·6% (HP), enriched with either fat (HP/F) or maize starch and sucrose (HP/C). Two groups received a diet with a low P:E ratio of 8·8% (LP), enriched with either fat (LP/F) or maize starch and sucrose (LP/C). The rate of weight gain on the HP diets was significantly greater than on the LP diets. There was no difference in urea production between any of the four diets: HP/F 1·23 (SE 0·12), HP/C 1·37 (SE 0·14), LP/F 1·64 (SE 0·22), LP/C 1·15 (SE 0·15) mmol nitrogen/kg per h. On the HP diets urea excretion was 0·77 (SE 0·07) mmol N/kg per h, 61% of production. There was significantly less urea excreted in the urine on diet LP/C than on LP/F (0·36 (SE 0·05) and 0·64 (SE 0·04) mmol N/kg per h respectively). A significantly greater percentage of the urea production was hydrolysed on the LP diets (61%) compared with the HP diets (39%), with the consequence that 50% of urea-N produced was available for synthetic activity on the LP diets compared with 30% on the HP diets. The increase in the urea hydrolysed on the LP diets was equivalent in magnitude to the decreased intake of N, so that overall intake plus hydrolysis did not differ between the LP and the HP diets. Crude N balance was similar on diets HP/F, HP/C and LP/C, but was significantly reduced on diet LP/F. These results show that there is an accommodation in urea kinetics during rapid catch-up weight gain, which becomes evident when the P:E ratio of the diet falls to 8·8%. It is proposed that, for a P:E ratio of 8·8%, protein is limiting for catch-up growth. When the intake has a P:E ratio of 8·8% the pattern of urea kinetics can be modified by the relative proportions of fat and carbohydrate in the diet. The measurement of urea kinetics provides a useful approach to the definition of the adequacy of the protein in the diet.

Urea kinetics: Growth: Undernutrition

During growth the pattern of nutrients required to satisfy the net deposition of balanced new tissue is different from that required for maintenance. The overall rate of catch-up growth, weight gain during recovery from severe malnutrition, is determined by the metabolizable energy intake (Waterlow, 1961), but for any given energy intake the relative proportions of fat or lean tissue deposited will be determined in part by the ability to synthesize lean tissue (Rudman *et al.* 1975). In man there is evidence that during rapid weight gain on a high-energy formula there is a tendency towards a relative preponderance of fat tissue deposition, with a limited gain of lean tissue (Jackson, 1990). There have been suggestions that limited deposition of lean tissue reflects an inappropriate intake of protein in either quality or quantity (MacLean & Graham, 1980).

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When the dietary intake of protein is inadequate to satisfy the metabolic demand there is an accommodation by the body. One aspect of the accommodation can be measured as an increase in the rate at which the urea produced by the liver is salvaged by the body as a potential source of nitrogen (Picou & Phillips, 1972; Jackson *et al.* 1980). The imbalance between intake and demand might be produced by a decrease in the intake of protein (Picou & Phillips, 1972; Jackson *et al.* 1980) or by an increase in the demand as in sickle-cell disease (Jackson *et al.* 1988). It can be shown that there is an increase in urea recycling during rapid growth, which would support the suggestion that protein intake may not be satisfying the demand at this time (Jackson, 1985).

Although changes in urea kinetics in response to changes in the balance between the intake of protein and the metabolic demand can be identified, no attempt has been made to define the nature of the relationship nor to explore the extent to which changes in urea kinetics are part of the mechanisms for adaptation to low-protein diets.

Understanding of the specific factors that control the rate of urea recycling is limited. It is known that the hydrolysis of urea is a function of the microflora of the lower bowel (Levenson *et al.* 1959), and that the flora ferment non-digestible carbohydrate as a source of energy (Cummings, 1981). We, therefore, speculated that the rate or efficiency with which urea hydrolysis and recycling takes place might be modulated by the quality of dietary energy, specifically the content of non-digestible carbohydrate. Studies in adults have shown that maize starch may be used as a source of non-digestible carbohydrate (Shetty & Kurpad, 1986).

In the present study we have looked at the effect of changes in the level of dietary protein and the quality of dietary energy on the rate of hydrolysis of urea and the utilization of urea-N in children gaining weight rapidly during catch-up growth from severe malnutrition. The findings support the contention that the intake of protein and the quality of dietary energy may exert independent effects on urea metabolism.

METHODS

Subjects

A total of twenty-six studies were carried out in twenty-four male children admitted to the metabolic ward of the Tropical Metabolism Research Unit, University of the West Indies, for the treatment of severe malnutrition. At the time of the study the acute phase of treatment had been successfully completed. All infections had been treated and the children were gaining weight on an *ad lib.* intake of a high-energy, milk-based formula. The studies were carried out during the period of rapid weight gain. The study had the approval of the Ethical Committee of the University Hospital of the West Indies, and the parents or guardians of each child gave written consent for the study to be conducted, without prejudice. The children received one of four diets for periods of up to 10 d. Each diet was given at a rate to provide 711 kJ (170 kcal) metabolizable energy/kg per d, and was adjusted on a daily basis for the child's actual weight. In preliminary studies it had become clear that the rate of weight gain of the children was one important variable that exerted an effect on the rate of urea hydrolysis and recycling (Jackson, 1985). In an attempt to minimize the effect of this variable a constant intake of energy was provided, to limit the range of weight gain from child to child. The children were fed equal quantities of food every 3 or 4 h, throughout the 24 h, for the duration of the study. The intake of each child was determined by weighing the cup before and after each feed, and any refusals were offered to the child at the end of each 24 h period. Generous supplements of minerals and vitamins were added to all the diets to ensure that they were unlikely to be limiting for rapid weight gain (Table 1, see p. 374). The children were weighed daily on an electronic balance,

and lengths were measured every week by experienced staff. Between the 7th and 10th day of each dietary period metabolic studies were carried out to measure urea kinetics. For this period of time the children received the feeds every 3 h.

Urea kinetics

Urea kinetics were measured by using a prime and intermittent oral doses of [$^{15}N^{15}N$]urea (97·5 atoms % excess, APC, France). A known weight of [$^{15}N^{15}N$]urea was made up in sterile water to a concentration of approximately 1 mg/ml. In preliminary studies it had been determined that following a priming dose equivalent to 12 h of intermittent oral doses, plateau levels of enrichment in urinary urea were reached within 6 h of the start of the study (Fig. 1). Intermittent doses of [$^{15}N^{15}N$]urea, 3 mg, were given every 3 h, for at least 24 h or until a complete 12 h urine had been collected satisfactorily, whichever was the longer. The solution of isotope was drawn up into a disposable syringe and placed in the back of the child's throat. With experience it was possible to ensure that all the dose was swallowed as given. The weight of the syringe was taken before and after dosing to demonstrate that the appropriate amount of isotope had been administered. Stools and urine were collected as described by Golden *et al.* (1981), a modification of the method of Liu & Anderson (1967). Urine was collected by continuous aspiration from a perineal urine bag. A sample of urine was collected before the administration of any isotope for the measurement of natural abundance. The volume of each specimen of urine was measured before being stored frozen in sealed vessels containing 0·5 ml 6 M-hydrochloric acid. Stools were collected into preweighed polyethylene bags attached to the perianal region. The times at which urine and stools were passed were recorded, as were any losses that occurred.

Diets

The composition of the four experimental diets, which were made up by weight, is shown in Table 1. The study was carried out in two stages. The high-energy diet that was used as a routine during the rehabilitation of the children on the ward was enriched with arachis oil to provide 5640 kJ (1350 kcal) and 31 g protein/kg. An attempt was made to formulate a carbohydrate-enriched formula on the same principles, but it was not possible to obtain a fluid formula of the same energy density. Therefore, the formulas were modified to produce a fat-enriched and a carbohydrate-enriched diet that would provide 4850 kJ (1160 kcal) metabolizable energy and 31 g protein/kg, 10·6% of energy from protein (diets HP/F and HP/C). These diets were given to fourteen children and represent study 1. No differences were determined overall in the urea kinetics between the two diets. In study 2 the formulas were further modified to ensure that the percentage of energy derived from protein was 8·8 (diets LP/F and LP/C), by adding arachis oil to the two diets used during study 1, to produce 5850 kJ (1400 kcal) metabolizable energy and 31 g protein/kg.

Study 1

Two groups of children received an energy-enriched diet that provided 711 kJ (170 kcal) metabolizable energy/kg per d and 4·5 g protein/kg per d, giving a high protein:energy (P:E) ratio of 10·6 when expressed as a percentage (HP diets). For one group the increased energy density was provided by the addition of arachis oil to the basal formula (HP/F), and in the other by the addition of maize starch and sucrose, (HP/C).

Study 2

Two groups of children received an energy-enriched diet that provided 711 kJ (170 kcal) metabolizable energy/kg per d and 3·7 g protein/kg per d, to give a low P:E ratio of 8·8% (LP diets). The increased energy was provided by either arachis oil (LP/F), or arachis oil

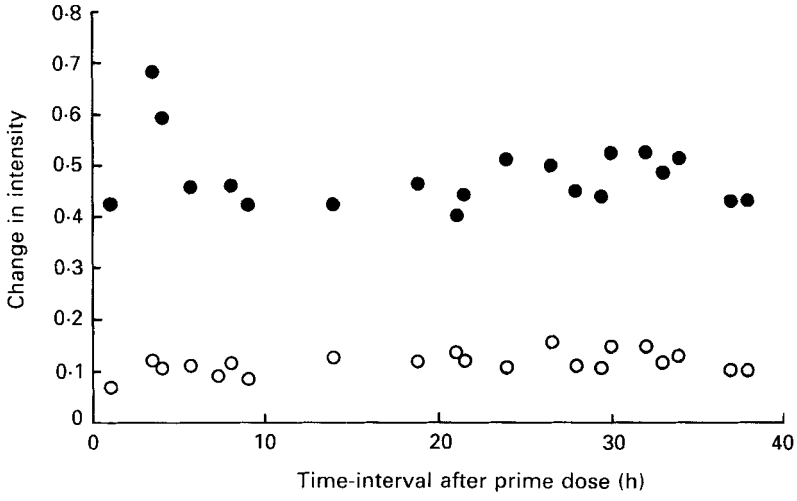

Fig. 1. Urea kinetics were derived, following the prime and oral presentation of [$^{15}N^{15}N$]urea, from the plateau level of labelling in urinary urea. The change in the intensity of the ratio at mass/energy (m/e) 29:28 (○) and m/e 30:28 (●) for N_2 derived from labelled urinary urea makes it possible to calculate urea kinetics (Jackson *et al.* 1984). Plateau is achieved within 6 h and maintained until the end of the study period for m/e 29:28 and 30:28-urea, derived from [$^{15}N^{14}N$]urea and [$^{15}N^{15}N$]urea respectively.

Table 1. *The composition (g/kg) of the four diets used in the study*

(Four diets were formulated to provide 711 kJ (170 kcal) metabolizable energy/kg per d. The two higher protein diets (HP) contained 10·6% of dietary energy from protein and were enriched with fat (HP/F) or carbohydrate (HP/C). The two lower protein diets (LP) contained 8·8% of dietary energy from protein and were enriched with either fat (LP/F) or carbohydrate (LP/C))

Diet... Ingredients	HP/F	HP/C	LP/F	LP/C
Pelargon powder	190	190	190	190
Arachis oil	33	—	60	27
Maize starch	—	25	—	25
Sucrose	—	50	—	50
Minerals and vitamins*	+	+	+	+
Metabolizable energy				
kJ	4870	4828	4931	5890
kcal	1165	1155	1419	1409
Protein (g)	31	31	31	31
Protein:energy ratio (%)	10·6	10·7	8·7	8·8

* For each day the supplement of vitamins and minerals provided: retinol 600 μg, thiamin 1·8 μmol, riboflavin 2·4 μmol, nicotinamide 54 μmol, vitamin D 10 μg, folate 11 μmol, potassium chloride 2 mmol/kg body-weight, magnesium chloride 1 mmol/kg body-weight, ferrous sulphate 18 μmol/kg body-weight, zinc acetate 8 μmol/kg body-weight, copper chloride 0·6 μmol/kg body-weight.

with maize starch and sucrose (LP/C). For two of the children it was possible to study the effect of both the diets during the period of rapid growth.

Analyses

The concentration of urea and ammonia in urine was measured using the Berthelot method (Kaplan, 1965), and urea-N was isolated for mass spectrometry using short ion-exchange column chromatography (Jackson *et al.* 1980). N_2 gas was liberated from urea by reaction

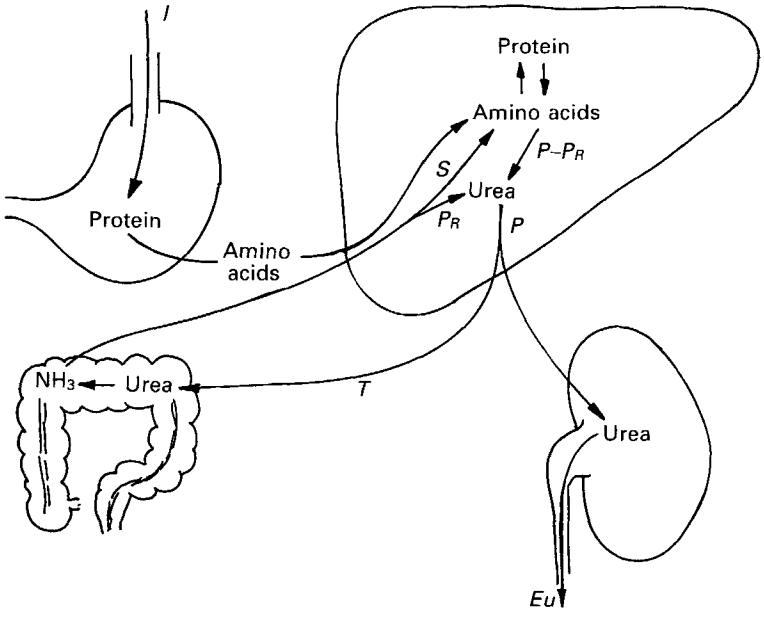

Fig. 2. The normal metabolic pathways for urea. Urea formation (P) takes place in the liver from dietary and endogenous amino acids. The urea is disposed of either by excretion in the urine (Eu) or by hydrolysis in the gastrointestinal tract (T). Nitrogen derived from urea hydrolysis in the bowel may either be re-formed into urea (P_R) or used for synthetic activities by being incorporated into amino acids and other compounds (S).

with alkaline hypobromite. In this reaction N is released from urea in a monomolecular reaction (Walser *et al.* 1954), hence the relative proportions of [$^{15}N^{15}N$]urea, [$^{15}N^{14}N$]urea and [$^{14}N^{14}N$]urea can be determined. Measurements were carried out in either a double collector (VG Micromass 602C) or a triple collector (SIRA 10, VG Isogas, Winsford, Cheshire) isotope ratio mass spectrometer. A technical error in the isolation of the stool samples meant that it was not possible to calculate the total N content of the stools, although isotopic enrichment could be measured.

Calculations and statistics

Urea kinetics were calculated by the model of Jackson *et al.* (1984) (Fig. 2). Ingested protein (I) is made available to metabolism as amino acids, which mix with the general body pool; amino acids also derive from protein degradation. Once an isotopic steady state has been achieved, the dilution of an intermittent dose of [$^{15}N^{15}N$]urea gives a measure of the rate of urea production in the body (P). A proportion of this urea is excreted in the urine (Eu). The difference between the urea produced and that excreted ($P-Eu$) is presumed to have been hydrolysed in the bowel (T), with the N being returned to the general metabolic pool. A part of this N is resynthesized to urea (P_R), whereas the remainder is available for synthesis into amino acids and proteins (S). The total urea produced comprises a component derived from the catabolism of dietary and endogenous amino acids, and a component (P_R) derived from urea hydrolysis in the bowel. The urea entering the bowel is doubly labelled, [$^{15}N^{15}N$]urea. The recycled urea (P_R) that is synthesized in the liver from $^{15}NH_3$, generated from urea hydrolysed in the bowel, will be singly labelled, [$^{14}N^{15}N$]urea. P_R is determined from measurements of singly labelled urea in the urine. The urea-N passes into a pool of metabolic N, and the flux through this pool (Q) can be derived. Details of the calculations are given in Jackson *et al.* (1984).

Comparisons between groups of data were carried out using Wilcoxon's Rank sum test.

RESULTS

A total of twenty-four children were entered into the study and altogether twenty-six studies were completed. Four of the studies were technically unsatisfactory, leaving twenty-two studies in twenty children. In consequence there were only four completed studies on diet HP/C with six completed studies on each of the diets HP/F, LP/F and LP/C. The clinical characteristics of the children who completed the study are shown in Table 2. The children were aged between 6 and 40 months. On admission six had a diagnosis of marasmus, five of kwashiorkor and nine of marasmic kwashiorkor (Wellcome Trust Working Party, 1970). The weight for age, an index of the degree of wasting, varied from 62 to 90%. The higher values were from children in whom oedema fluid would have given a false impression of the degree of wasting at admission. The degree of stunting, height for age, varied from 75 to 95% of a reference standard.

All the studies were carried out while the children were gaining weight rapidly. The diets were readily accepted and all the children for whom values are reported took all the food offered for the duration of the study. As can be seen from Table 3, the rate of weight gain was significantly different on HP diets (19 g/kg per d) compared with the LP diets (13 g/kg per d) ($P < 0{\cdot}01$). For the individual children the rates of weight gain varied from 9 to 28 g/kg per d, all substantially greater than the normal rate of weight gain for children of this age (up to 2 g/kg per d). Crude N balance ($I-Eu$) was significantly reduced on LP/F, compared with LP/C or either of the two HP diets.

Aspects of urea kinetics were calculated from the plateau enrichment in urinary urea. One of the limitations of giving a priming dose of label to shorten the time taken to achieve plateau is that one has to have some idea of the rate of urea production in order to be able to choose a suitable priming dose. We had defined suitable conditions in a series of preliminary studies, and for all the values reported here it was considered that a satisfactory plateau had been achieved by visual inspection (an example is shown in Fig. 1). For all children at least six points were available at plateau, and for some considerably more.

Study 1

The complete experimental schedule was completed in six children on diet HP/F and four children on diet HP/C. There was no significant difference between the two diets with respect to rate of weight gain (HP/F 18 (SE 2·2) and HP/C 20 (SE 3·2) g/kg per d) or urea kinetics. Urea production (P) was similar for both groups (HP/F 1·23 (SE 0·12), HP/C 1·37 (SE 0·14) mmol N/kg per h), and represented 59% of N intake. Eu was 61% of P, and was not significantly different between the two diets (HP/F 0·71 (SE 0·12) and HP/C 0·83 (SE 0·02) mmol N/kg per h). There was no significant difference in T between the two groups (HP/F 0·45 (SE 0·05) and HP/C 0·54 (SE 0·15) mmol N/kg per h), nor in S (HP/F 0·37 (SE 0·05) and HP/C 0·37 (SE 0·08) mmol N/kg per h). Hence of the 39% of P that was hydrolysed in the bowel, T, 30% of the N was available for further metabolic interaction. As no difference was found for any measurement between the groups on the two HP diets, the results were pooled for further analysis (Tables 3 and 4).

Study 2

The schedule was completed for six children on each of the low-protein diets (LP/F and LP/C). There was no significant difference between the two groups in the rate of weight gain (Table 3). The difference in P between the two groups (LP/F 1·64 (SE 0·22), LP/C 1·15

Table 2. *The ages of the children on admission to the ward, the anthropometry and diagnosis at admission and the weight at the time of the study*

(The weight-for-age (wt/age), weight-for-height (wt/ht) and height for age (ht/age) have been calculated from the National Center for Health Statistics standards (Hamill *et al.* 1979). The diagnosis at admission is based on the Wellcome classification (Wellcome Trust Working Party, 1970))

	Subject	Age (m)	Wt (kg)	Ht (m)	Wt/age (%)	Wt/ht (%)	Ht/age (%)	Diagnosis	Wt at study (kg)
					Dietary treatment*: HP/F				
	1	11	5·42	0·705	56	62	95	M–K	6·77
	2	14	6·50	0·71	62	70	90	K	8·23
	3	15	4·14	0·605	39	71	75	M	5·23
	4	13	7·90	0·71	77	85	93	K	7·38
	5	14	6·01	0·705	57	65	95	M	7·54
	6	40	8·38	0·76	55	82	78	M–K	9·84
Mean		17·8	6·06	0·699	—	73	—	—	7·50
SD		—	1·2	0·05	—	—	—	—	1·5
					HP/C				
	7	11	4·73	0·60	49	88	80	M	5·12
	8	6	4·41	0·57	58	90	80	M–K	5·20
	9	8	5·61	0·66	65	78	94	K	6·96
	10	10	3·80	0·59	40	70	80	M	5·96
Mean		8·8	4·63	0·605	—	82	—	—	6·50
SD		—	0·75	0·04	—	—	—	—	1·4
					LP/F				
	11	15	5·92	0·675	55	79	76	M–K	6·80
	12	26	7·23	0·755	56	72	84	M–K	8·90
	13/B	21	5·25	0·645	44	74	75	M–K	6·76
	14/A	7	4·78	0·595	59	80	87	M–K	5·38
	15	24	6·45	0·69	51	75	78	M	7·56
	16	6	3·62	0·58	48	70	88	M–K	4·61
Mean		16·5	5·54	0·657	—	75	—	—	6·67
SD		—	1·3	0·06	—	—	—	—	1·5
					LP/C				
	13/A	21	5·25	0·645	44	74	75	M–K	6·07
	17	16	6·36	0·69	58	70	86	M	7·84
	14/B	7	4·78	0·595	59	80	87	M–K	6·19
	18	12	7·23	0·705	72	80	93	K	8·07
	19	19	6·38	0·725	55	69	86	M–K	7·78
	20	11	6·43	0·69	66	78	92	K	7·68
Mean		14·3	6·07	0·675	—	75	—	—	7·27
SD		—	0·9	0·05	—	—	—	—	0·9

M, marasmus; K, kwashiorkor; M–K, marasmic kwashiorkor; HP/F and HP/C, protein:energy ratio (P:E) 10·6%; LP/F and LP/C, P:E 8·8%.

* For details, see Table 1 and p. 373.

(SE 0·15) mmol N/kg per h) did not reach statistical significance. There was a significant difference in *Eu* between the dietary groups (LP/F 0·64 (SE 0·04) and LP/C 0·36 (SE 0·05) mmol N/kg per h) ($P < 0·01$). However, *Eu*/*P* was not statistically different between diet LP/C (33%) and diet LP/F (44%). Although *S*/*P* was greater on diet LP/C (58) than on diet LP/F (42), this difference did not reach statistical significance. Similarly there was no statistical difference in *S*/*T* (LP/C 86 and LP/F 76).

Table 3. *Urea kinetics (mmol nitrogen/kg per h) in four groups of young children during rapid catch-up weight gain and given diets with a protein:energy ratio of 10·6 % (high protein (HP)) or 8·8 % (low protein enriched with fat (LP/F) or carbohydrate (LP/C))*

(Values are means with their standard errors)

Dietary treatment*...	HP		LP/F		LP/C	
	Mean	SE	Mean	SE	Mean	SE
Wt gain (g/kg per d)	19[a]	1·8	14[ab]	1·2	12[b]	1·4
I	2·19		1·76		1·76	
P	1·28[a]	0·09	1·64[a]	0·22	1·15[a]	0·15
$P-P_R$	1·13[a]	0·07	1·38[a]	0·13	1·04[a]	0·13
Eu	0·77[a]	0·07	0·64[a]	0·04	0·36[b]	0·05
T	0·49[b]	0·05	1·03[a]	0·24	0·80[a]	0·16
P_R	0·12[a]	0·04	0·26[a]	0·11	0·10[a]	0·02
S	0·37[b]	0·04	0·77[a]	0·16	0·69[a]	0·15
$T+I$	2·68[a]	0·07	2·76[a]	0·24	2·55[a]	0·16
$S+I$	2·56[a]	0·05	2·50[a]	0·15	2·45[a]	0·15
Q	4·00[a]	0·07	4·52[a]	0·98	5·17[a]	0·94
$I-Eu$	1·42[a]	0·12	1·12[b]	0·04	1·40[a]	0·05

I, dietary intake of N; P, rate of urea production; Eu, rate of urea excretion in urine; T, rate of urea hydrolysis in the gastrointestinal tract; P_R, rate of urea synthesis from N deriving from urea hydrolysis in the gastrointestinal tract; S, rate of movement of N from hydrolysed urea into synthetic metabolic pathways; Q, rate of flux of N through the pool into which N from hydrolysed urea passes.

[a,b] Values in horizontal rows with different superscript letters were significantly different ($P < 0·05$).

* For details, see Table 1 and p. 373.

Table 4. *Urea kinetics (mmol nitrogen/kg per h) in four groups of young children during rapid catch-up weight gain and given diets with a protein:energy ratio of 10·6 % (high protein (HP)) or 8·8 % (low protein enriched with fat (LP/F) or carbohydrate (LP/C)). The relative rates of different components of urea kinetics are expressed as percentages*

Dietary treatment*...	HP	LP/F	LP/C
P/I	59[a]	93[b]	62[a]
$P/T+I$	47[a]	58[b]	43[a]
Eu/I	35[a]	36[a]	20[b]
Eu/P	61[a]	44[ab]	33[b]
$Eu/T+I$	29[a]	24[a]	15[b]
T/I	22[b]	57[a]	45[a]
T/P	39[a]	56[ab]	67[b]
T/Q	14[a]	26[a]	16[a]
P_R/T	24[a]	24[a]	14[a]
S/I	17[b]	42[a]	39[a]
S/P	30[a]	42[ab]	58[b]
S/T	76[a]	76[a]	86[a]
$S/T+I$	14[b]	25[a]	25[a]

I, dietary intake of N; P, rate of urea production; Eu, rate of urea excretion in urine; T, rate of urea hydrolysis in the gastrointestinal tract; P_R, rate of urea synthesis from N deriving from urea hydrolysis in the gastrointestinal tract; S, rate of movement of N from hydrolysed urea into synthetic metabolic pathways; Q, rate of flux of N through the pool into which N from hydrolysed urea passes.

[a,b] Values in horizontal rows with different superscript letters were significantly different ($P < 0·05$).

* For details, see Table 1 and p. 373.

Comparison of study 1 and study 2

When the HP diets were compared with the LP diets there were significant differences in *Eu*, *T* and *S*. There was no difference in *P* between the two levels of protein (HP 1·28 (SE 0·09) and LP 1·39 (SE 0·15) mmol N/kg per h). On the HP diets *Eu* (0·77 (SE 0·07) mmol N/kg per h, *Eu*/*P* 62%) was significantly greater than on the LP diets (0·50 (SE 0·05) mmol N/kg per h, *Eu*/*P* 38%) ($P < 0·01$). The significant decrease in *Eu* on the LP diets was accounted for mainly by a highly significant decrease on LP/C. There was a significant increase in *T* on the LP diets (HP diets 0·49 (SE 0·07) mmol N/kg per h, *T*/*P* 38% and LP diets 0·91 (SE 0·14) mmol N/kg per h, *T*/*P* 62%) ($P < 0·01$). The magnitude of the increase in *T* on the LP diets was almost identical to the overall reduction in *I* on these diets, with the consequence that no difference could be demonstrated for $T+I$ between the two groups (HP 2·68 (SE 0·07) and LP 2·66 (SE 0·14) mmol N/kg per h). *S* was significantly greater on the LP diets (HP 0·37 (SE 0·04) and LP 0·73 (SE 0·10) mmol N/kg per h; $P < 0·01$).

The most efficient use of the available N was seen on diet LP/C. The finding that *P* was lowest on diet LP/C makes it difficult to interpret the importance of absolute changes in *Eu* and *T*. However, *Eu* was also significantly reduced in relative terms (*Eu*/*P* 33%) compared with diets HP/F (60%) and HP/C (63%), demonstrating more effective retention and hydrolysis of urea-N on diet LP/C. In both absolute and relative terms the disposal of *T* into *S* was greater on diet LP/C than on diets HP/F and HP/C. Despite a significantly lower $P/T+I$ on diet LP/C than on LP/F, *S* was not different between the two diets.

DISCUSSION

In designing a study of this kind a number of factors have to be taken into consideration. An extensive series of preliminary studies had made it quite clear that the metabolic demand for protein may vary from child to child, and the most important determinant of the variability appeared to be the dietary energy intake (Jackson, 1985). Therefore it was necessary to choose a level of intake that was sufficiently high to allow for substantial catch-up weight gain, but was at a level of intake that could be achieved readily by most children. Based on experience, an intake of 711 kJ (170 kcal)/kg per d was selected. Although children recovering from malnutrition can ingest considerable volumes of food, bulk may play a role in limiting intake (Ashworth, 1975). This was made obvious in the formulation of a food rich in carbohydrate, where the bulk and consistency of the food limit the energy density and the intakes that can be realized. These factors determined the upper limit to the maize starch additions, and the ultimate composition of the intakes.

Protein intake

All the children received a diet that provided the same intake of energy (711 kJ (170 kcal)/kg per d). Although all the children gained weight at a rate significantly greater than normal, there was a significant difference in the rate of weight gain between the two levels of protein intake. On the assumption that the energy requirements for maintenance were not substantially different between the two diets, a cost of growth analysis (Jackson *et al.* 1977) would indicate that a greater proportion of lean tissue was being deposited on the HP diets (66%) than on the LP diets (38%). These results would suggest that although the overall rate of weight gain might be determined by the dietary energy (Waterlow, 1961), protein might become limiting for lean tissue deposition (MacLean & Graham, 1980) when the percentage of dietary energy derived from protein falls below 9–10%. On the basis of

the weight changes it is reasonable to presume that the demand for protein was not being satisfied by the dietary intake on the LP diets. Even though the absolute intake of protein was in excess of normal requirements, the intake of protein relative to the need created by an increased intake of energy was inadequate. Nevertheless, crude nitrogen balance ($I-Eu$), was not different between the HP diets and diet LP/C, although there was a significant reduction in $I-Eu$ on LP/F. However, caution should be used in over-interpreting the results of crude N balance. As it is known that maize starch might increase faecal losses of N (Shetty & Kurpad, 1986), extrapolation from crude balance to N balance may not be justified.

Urea production

The assumption is made frequently that urea excretion and urea production are synonymous. This assumption is incorrect and unjustified, and the use of urea excretion to approximate urea production can lead to erroneous conclusions. In vitro and in vivo studies have provided a body of evidence to show that urea production may respond to two aspects of the dietary intake of protein: the amount of protein and the balance of amino acids within the protein. Urea production increases when the intake of amino acids is unbalanced, as seen with a protein of poor quality (Harper *et al.* 1970). In the present study there was no significant difference in P between any of the diets. It is surprising that P was not significantly reduced on the low-protein diet. On diet LP/C there was a non-significant reduction in P as might have been expected, but this was offset by a non-significant increase on diet LP/F. Whether these changes have any biological significance requires further study. It may be that the rate of urea production by the liver is responsive to the total flux of N up the portal tract ($I+T$), rather than the dietary intake alone. Therefore, it is of note that the reciprocal change in I and T from the HP to the LP diets maintained $I+T$ constant.

Urea excretion

The urinary excretion of urea is responsive to the dietary intake of protein, and changes in the rate of urea excretion represent the primary mechanism through which N balance is maintained. The disposal of the urea produced may vary with the level of intake. On an adequate intake of protein about two-thirds of the urea is excreted, with one-third being retained by the body (Walser & Bodenlos, 1959). On a low-protein intake only one-third is excreted, with two-thirds being retained (Picou & Phillips, 1972). Although no studies have been carried out to define precisely the point of cross-over, the available information suggests that this might be in the region of the maintenance requirement for protein, i.e. at the point where the intake of protein ceases to satisfy the metabolic demand (Jackson, 1983). The finding that about 60% of the urea produced was excreted on both HP diets implies that protein intake was adequate. The addition of fat to each of these diets diluted the protein content from 10·6 to 8·8% of dietary energy. The consequence was a marked shift in the disposal of the urea produced, with only 30–40% being excreted. This resulted in an absolute increase in T as well as a relative increase in T/P. These observations provide metabolic evidence that the protein intake on the LP diets was relatively inadequate. There was a significant difference between the two LP diets in the amount and the proportion of urea excreted, with relatively more being excreted on diet LP/F than on diet LP/C.

Urea-N utilization

The corollary of a decrease in the proportion of urea excreted on the LP diets is an increase in the proportion retained and hydrolysed. Increased retention of urea-N was found on the LP diets relative to the HP diets, with the highest retention on diet LP/F. Despite the

finding that T was greater on diet LP/F than on diet LP/C (because about one-quarter of T returned to P_R on diet LP/F), the overall retention of urea-N was not different between the two LP diets (S/P about 50%). As noted, the absolute increase in T on the LP diets was similar in magnitude to the reduction in N intake relative to the HP diets. Overall $I+T$ was unchanged, with the effect that $S/I+T$ was significantly greater on the LP than the HP diets. Hence there was little difference in the total N available for metabolism ($I+S$) between the HP and the LP diets.

Dietary energy

One of the objectives of the study was to explore the effect that changes in the source of dietary energy would have upon urea kinetics. The results show that no difference could be discerned on the HP diets, and although an influence may be exerted on the LP diets this is small relative to the overall effect of the P:E ratio. In consequence, most of the changes in urea kinetics that have been measured may be accounted for by the relative contribution of protein to the total energy content of the diet, with a smaller contribution coming from the relative fat or carbohydrate content. A reduction in the percentage of energy derived from protein from 10·6 to 8·8 resulted in a slower rate of weight gain, a higher energy cost of weight gain with the presumed deposition of less lean tissue, and a metabolic response which characterizes adaptation to a low-protein intake. When the percentage of dietary energy derived from protein was 10·6, no difference was discerned between HP/F or HP/C diets. However, at a 'marginal or deficient' intake of protein, providing only 8·8% of dietary energy, metabolic differences began to emerge. The differences identified between the diets may be attributed to a difference in the source of energy in the diet. Limited support was obtained for the original hypothesis that urea-N would be utilized with increased efficiency when the diet contained more non-digestible carbohydrate, which could act as a source of energy for the gastrointestinal microflora. It was hypothesized that by increasing the metabolic activity of the microflora the demand for N would increase, thereby stimulating an increase in urea hydrolysis. The demonstration that urea excretion was increased and crude N balance ($I-Eu$) decreased on diet LP/F relative to diet LP/C is supportive of the hypothesis. However, the absolute retention of urea-N (S) was greatest on diet LP/F (Fig. 3). The explanation of the basis of these differences is central to our overall understanding of urea kinetics.

The differences in composition between the two LP diets were not as large as originally planned, because of the difficulties in making a carbohydrate-rich formula which could be taken easily by the children. It may be that the observed differences between the two diets may be of biological relevance, with implications for diets that vary more extensively in their fat and carbohydrate composition. On the LP diets, the demand for N demonstrably exceeded the supply, and urea-N was salvaged in increased amounts from the colon. Any differences in P between diet LP/F and diet LP/C can only be explained on the basis of protein quality. Protein quality is an expression which relates the pattern of amino acids in the dietary protein to the pattern required for the synthesis of proteins required by the body. Differences in P would indicate either that there is a substantially different pattern of proteins being formed on diet LP/F compared with diet LP/C, or that the pattern of amino acids available from the two diets to form a similar pattern of proteins is different. We have no way of differentiating the two possibilities from the results of the present study. There is considerable controversy about the form in which N derived from urea hydrolysis is made available to the body. Moran & Jackson (1990) have shown that when urea-N was instilled into the colon of adults about 6% was absorbed intact, with the remainder being rapidly hydrolysed. They concluded that following hydrolysis, between 15 and 20% of the dose had been absorbed as ammonia, with the majority of the dose, 70–80%, being fixed

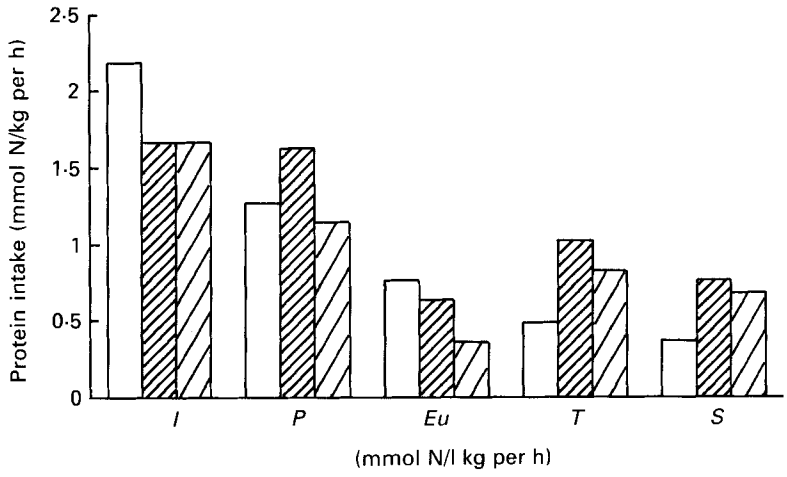

Fig. 3. Urea kinetics were measured, during rapid catch-up weight gain, in four groups of children who had been severely malnourished. All the children received 711 kJ (170 kcal)/kg per d for 10 d and urea kinetics was measured using prime and intermittent oral doses of [$^{15}N^{15}N$]urea. One group received a diet with a high protein:energy ratio of 10·6%, (HP, □). Two other groups received a diet with a low protein:energy ratio of 8·8%, enriched with either fat (LP/F, ▨) or carbohydrate (LP/C, ▨). The dietary intake of protein (*I*) is expressed as mmol N/kg per h, as are *P*, *Eu*, *T* and *S* (for details see Fig. 2).

by the microflora before retention by the body in a functionally useful form. The suggestion has been made that urea-N can be utilized by the microflora for the synthesis of essential and non-essential amino acids which are available to the host by absorption (Tanaka *et al.* 1980; Jackson, 1983). One interpretation of the differences between diets LP/F and LP/C may be that on LP/F the stimulus for increased urea hydrolysis was present, but the ammonia produced was not fixed in a functionally useful form as effectively as on diet LP/C. Although it has been shown that absorbed ammonia passing up the portal tract is preferentially utilized for urea synthesis (Nissim *et al.* 1981), it is less clear how the portal ammonia is handled in an individual on a low-protein intake. In order to be able to carry understanding of this point forward, a clearer definition is required of the way in which urea-N is metabolized following hydrolysis in the bowel in individuals with different nutritional states on a range of diets. Overall the re-utilization of urea-N was not different on the two LP diets, but the relative disposal of the retained urea-N on diet LP/F showed a greater proportion being returned to urea synthesis. Thus, the ratio P_R:*S* on diet LP/F was 0·34 (SE 0·09), not different from that on the HP diet (0·34, SE 0·09), but twice as great as that for LP/C (0·16, SE 0·03) although the difference did not reach statistical significance. In general, the indications were that there was more efficient use of N on diet LP/C. On diet LP/F urea production was increased, urea hydrolysis was increased and there was an increase in the return of N from urea hydrolysis to urea synthesis, giving all the appearance of a 'futile cycle' as described by Walser (for discussion, see Jackson *et al.* 1981). In contrast, on diet LP/C urea production was low, with little excretion, and there was effective utilization of N coming from urea hydrolysis for synthetic activity.

Adaptation

It may be possible to compare the results of the present study with those obtained by Picou & Phillips (1972). Although the children they studied were said to be either malnourished or recovered from malnutrition, they were given one of two diets that provided 669 kJ (160 kcal)/kg per d and either 1·14 or 3·7 g protein/kg per d, with a P:E ratio of 2·9 and

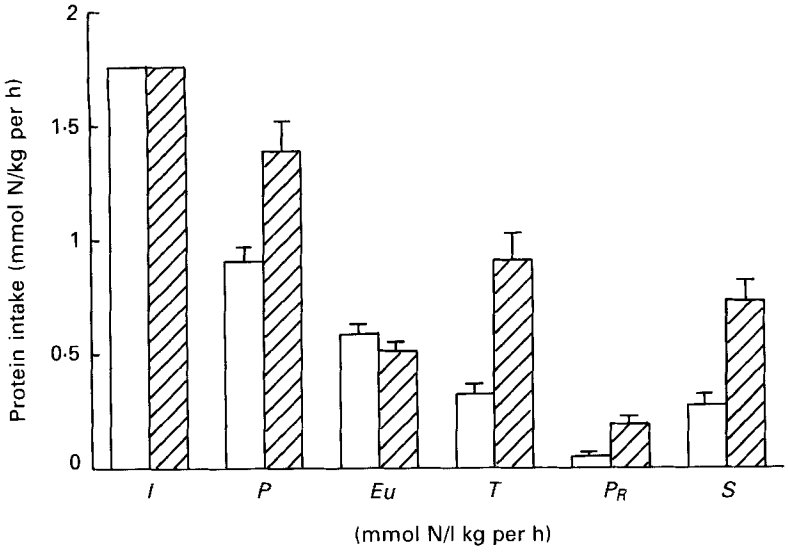

Fig. 4. A comparison is drawn between the urea kinetics measured by Picou & Phillips (1972) (□), and those obtained in the present study (▨). Both studies were carried out on children who had been hospitalized for severe malnutrition. In both studies the children received a milk-based diet that provided 3·7 g protein/kg per d. In the study of Picou & Phillips (1972) the diet provided 669 (160 kcal)/kg per d, a protein:energy (P:E) ratio of 9·25 %, whereas in the present study the provision of 711 (170 kcal/kg per d gave a P:E ratio of 8·8 %. The findings show that with a P:E ratio of 9·25 %, *P* was significantly lower ($P < 0·01$), and *Eu*/*P* was greater than with a P:E ratio of 8·8 %. *T* and *S* were significantly greater with a P:E ratio of 8·8 % ($P < 0·01$, Wilcoxon Rank sum test), than with a P:E ratio of 9·25 %. Values are means with their standard errors represented by vertical bars. The dietary intake of protein (*I*) is expressed as mmol N/kg per h, as are *P*, *Eu*, *T*, P_R and *S* (for details see Fig. 2).

9·25 % respectively. On this level of energy intake all the children would have been expected to gain weight. The differences in urea kinetics between the malnourished and recovered children were small, and the main changes could be attributed to the level of dietary protein. On the higher-protein intake the source and the absolute amount of protein were identical to the intake of protein on LP diets in the present study. The difference between the two studies was an energy intake of 42 kJ (10 kcal)/kg per d. A comparison of the results of the urea kinetics from the two studies is shown in Fig. 4. With a P:E ratio of 9·25 % the pattern of urea kinetics is similar to that obtained on an adequate protein intake; in contrast, on a P:E ratio of 8·8 % the pattern has shifted to that obtained in the adapted state – a decrease in *Eu*/*P* and an increase in *T*/*P*. The implication is that the metabolic demand presented by an additional 42 kJ (10 kcal) energy/kg is sufficient to tip the balance from an adequate to an inadequate protein intake. One important observation by Picou & Phillips (1972) was that the relative disposal of urea-N by the body changed substantially when moving from a higher to a lower protein intake. To an extent this observation has been overlooked because on the lower protein intake there was a marked absolute fall in *P*, with the overall consequence that although *T*/*P* increased there was in fact an absolute reduction in *T*. The P:E ratio of the lower protein diet used by Picou & Phillips (1972), 2·9 %, was that required for N balance, at a maintenance level of energy intake (Chan & Waterlow, 1966) (about 420 kJ (100 kcal)/kg per d). The provision of this diet at 669 kJ (160 kcal)kg per d by Picou & Phillips (1972) is likely to have induced a significant metabolic stress (MacLean & Graham, 1980). In the present study, providing a P:E ratio of 8·8 % was sufficient to stimulate the adaptive response, with a greater proportion of the

production of urea-N being retained. However, because P was maintained at a relatively high level, T and S were increased in absolute terms also, implying that the response was adaptive and purposive.

Taken together, the two studies give some indication as to the reason why N balance might be a relatively insensitive tool for assessing the requirements for protein. The results suggest that the body is able to switch on the mechanisms for adapting to relatively low intakes of protein in response to small changes in dietary intake. The effective salvaging of urea-N through the bowel serves to offset the impact of a reduced N intake, making it more difficult to define the point of cross-over in a balance experiment. If this interpretation is correct the findings of the present study are of potential importance not only in the definition of protein requirements, but also for our appreciation of fundamental aspects of N metabolism. The findings suggest that the measurement of urea kinetics may be a useful and sensitive method for assessing the adequacy of both the quantity and the quality of the dietary protein intake. If there is substantial hydrolysis and recycling of urea-N through the bowel, it is not possible to consider protein quality simply in terms of the dietary intake without giving due consideration to the form in which the urea-N obtained from the salvage system is made available to metabolism.

The present work was designed to demonstrate that one of the major determinants which might influence urea hydrolysis and recycling was the form in which energy is taken in the diet. The results show that the dominant influence on urea hydrolysis appears to be the balance between the supply of dietary protein in relation to the demand. By comparison the influence exerted by changes in the form in which dietary energy was provided was relatively small.

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